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The traditional Hungarian medicinal plant *Centaurea sadleriana* Janka accelerates wound healing in rats

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ABSTRACT

Ethnopharmacological relevance: The aerial parts of *Centaurea sadleriana* Janka, a species native to Hungary, have been used for the healing of wounds of livestock in Hungarian folk medicine. This is the first report of the ethnomedicinal use of this plant.
Aim of the study: This study was aimed at investigating the wound-healing efficiency of different extracts of *Centaurea sadleriana*.
Materials and methods: Experimental wounds inflicted on healthy rats by means of a branding iron were treated topically with different extracts and fractions of extracts of the aerial parts of *Centaurea sadleriana*. To assess the effectiveness of treatment, an absolute control (no treatment), a vehicle control (Carbomer gel) and a positive control group (1% salicylic acid in Carbomer gel) were applied.
Results: The *n*-hexane fraction of the methanol extract significantly accelerated the wound-healing process. This effect was rather similar to that of the positive control gel. Other fractions exhibited more moderate activities.
Conclusions: The apolar fraction of the methanol extract of *Centaurea sadleriana* facilitated wound healing significantly, corroborating the folk medicinal use of this plant.

1. Introduction

Despite the considerable advances in the pharmaceutical industry, the availability of drugs capable of stimulating the process of wound healing is still limited. Only 1–3% of the drugs listed in Western pharmacopoeias are intended for use on the skin or wounds; by comparison, at least one-third of herbal remedies are applied in this way (Kumar et al., 2007). The scientific assessment of ethnopharmacological data may result in the discovery of novel, reasonably applicable medicinal plants to treat wounds (Akkol et al., 2009a; Kokane et al., 2009). Moreover, medicinal plants are rich sources of new efficacious wound-healing substances (Blazsó et al., 2004).

The genus *Centaurea* (Asteraceae) comprises some 300–350 species, 221 of which are native to Europe (Tutin et al., 1976). In Hungary, 22 species are native (Simon, 2000). The species of the genus are not widely applied in European folk medicine, though there are some references to their usage, most frequently for the treatment of ophthalmia (*Centaurea calcitrapa* L., *Centaurea cyanus* L.), fever (*Centaurea cyanus*, *Centaurea jacea* L., *Centaurea solstitialis* L.), gynecological problems (*Centaurea cyanus*), digestive complaints (*Centaurea calcitrapa*, *Centaurea cyanus*), wounds and dermatological complaints (*Centaurea calcitrapa*, *Centaurea cyanus*, *Centaurea jacea*) (Kern et al., 1972; Hänsel et al., 1992). In Hungarian traditional medicine, the most frequently used species is *Centaurea cyanus*, presumably because of its wide-ranging geographical distribution. The majority of the Hungarian *Centaurea* species have been poorly analyzed from phytochemical and pharmacological aspects.

The most characteristic compounds identified in *Centaurea* are sesquiterpene lactones of germacranolide, elemanolide (Wagner, 1977), guaianolide (Akkol et al., 2009b) and eudesmanolide type (Fortuna et al., 2001). Further important and characteristic classes of compounds in this genus are polyacetylenes, flavonoids (flavones, flavonols, flavanones and flavanonols) and other phenolic compounds (characteristically cinnamic acid derivatives) (Wagner, 1977).

The medicinal application of *Centaurea sadleriana* Janka, a herbaceous plant endemic in the Carpathian Basin (Hungary, eastern Austria and Slovakia), has not been documented previously. The ethnomedicinal application of *Centaurea sadleriana* was brought to our attention by Ferencné Szabó (Jakabszállás, Bács-Kiskun County, Hungary) in 2006: in the region of Jakabszállás the decoction of the aerial parts of this plant has been used topically for the treatment of wounds of livestock (especially sheep). Phytochemical and

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pharmacological studies on this plant have not been performed so far.

The process of wound healing is in general divided into three phases: the inflammatory phase, the proliferation phase and the remodeling phase (Yamaguchi and Yoshikawa, 2001). The inflammatory phase commences shortly after the injury, while the migratory and proliferative process begins within a few days and involves the major processes of healing. The remodeling process lasts for up to a year and is responsible for scar tissue formation and the development of new skin (Hunt et al., 2000).

In some cases, herbal remedies that have a direct wound-healing effect also possess anti-inflammatory activity. As the reduction of inflammation shortens the inflammatory period, herbal remedies with a topical anti-inflammatory effect may accelerate wound healing; however, antiphlogistic activity is not a prerequisite of this wound-healing effect. The documented anti-inflammatory effectiveness of several *Centaurea* species (*Centaurea hierapolitana* L., *Centaurea calolepis* L., *Centaurea cadmea* L., *Centaurea cyanus*, *Centaurea chilensis* Hook. et Arn.) (Negrete et al., 1993; Garbacki et al., 1999; Karamenderes et al., 2007) has been confirmed by *in vitro* and animal experiments, but the exact mechanisms of action have not been elucidated. As active agents, sesquiterpene lactones (Negrete et al., 1993; Yesilada et al., 2004) and polysaccharides (Garbacki et al., 1999) have been identified. Other compounds that may exert an anti-inflammatory effect are polyacetylenes, since some natural compounds of this type of exhibit antiphlogistic activity (Redl et al., 1994).

Herbal extracts with an antimicrobial effect may also shorten the healing period by preventing the infection of the wound. The antibacterial and antifungal activities of *Centaurea* species have been attributed to their sesquiterpene lactone content or to the volatile compounds of the plants, as demonstrated by a series of experiments (Vajs et al., 1999; Barrero et al., 2000; Skaltsa et al., 2000; Saroglou et al., 2005; Skliar et al., 2005; Bulent Kose et al., 2007).

Efforts to influence the proliferative and remodeling phases of wound healing are important elements of research into wound-healing activity and a promising area in the field of therapy. The wound-healing activity of certain plants containing polysaccharides (e.g. *Aloe barbadensis* L.) has been clinically confirmed, and it is assumed that this activity is closely related to the influence on the immune system (Zhang and Tizard, 1996). In one experiment, the polysaccharide fraction of *Centaurea cyanus* did prove to interfere with the immune system, besides inhibiting inflammation (Garbacki et al., 1999).

The potential wound-healing and anti-inflammatory effects of the majority of the *Centaurea* species native to Hungary have not been investigated scientifically so far. The objective of the present work was an *in vivo* evaluation of the postulated wound-healing effect of *Centaurea sadleriana*.

2. Materials and methods

2.1. Plant material

The aerial parts of *Centaurea sadleriana* Janka were collected in the region of Jakabszállás, Hungary in June 2007 and authenticated by Tünde Ludnai (Kiskunság National Park, Kecskemét, Hungary). A voucher specimen (No. CE-001) has been deposited in the Herbarium of the Institute of Pharmacognosy, University of Szeged, Szeged, Hungary.

2.2. Preparation of the plant extracts

The plant material was air-dried and ground to obtain a coarse powder. 40 g of the ground material was extracted with 200 mL of boiling water for 10 min in an ultrasonic bath, and the extract was lyophilized (this material was denoted *Centaurea sadleriana* extract 1, CSE-1; 4.3 g). In the next experiment, 150 g of the ground material was extracted with 5 L of methanol in a percolator. One-fifth of this extract was evaporated in vacuum (CSE-2; 2.8 g). The remaining methanol extract was concentrated to 250 mL, diluted with water to 500 mL and partitioned with *n*-hexane (3× 250 mL) and then chloroform (3× 250 mL). The *n*-hexane (CSE-3; 1.4 g) and chloroform phases (CSE-4; 1.2 g) and the residue of the methanol extract after extraction with *n*-hexane and chloroform (CSE-5) were evaporated in vacuum.

2.3. Animals and experimental wounds

Wound formation was carried out using a well-established method as described previously (Blazsó et al., 2004). Briefly, female Sprague–Dawley rats weighing 140–160 g were depilated (Veet depilator cream, Reckitt Benckiser) on an area on the back measuring approximately 30 cm^2. Under isoflurane (2.5%) narcosis, wounds were elicited symmetrically on six different depilated areas through contact with a 100 °C branding iron (r = 4 mm) for 5 s. One day after this procedure, a crusting process was observed. The wound-healing time was given as the number of days required for 50% of the scabs to separate spontaneously from the animals. Separation of the scabs was always examined at the same time of day. The rats were kept at controlled ambient temperature (22.0 ± 1.0 °C) under a 12-h light and 12-h dark schedule. Rat chow and tap water were supplied *ad libitum*. The experiments were conducted in full accordance with the institutional guidelines of the University of Szeged, Hungary and with the international rules concerning animal experiments and biodiversity rights. The study was approved by the Committee on Animal Research at the University of Szeged, Hungary (IV/02867-2/2008).

2.4. Treatment of wounds

The different plant extracts were suspended at a concentration of 2.5% in a gel composed of 0.9% Carbomer 934 P, 1% NaOH solution (30%) and 98.1% purified water. 300 mg of Carbomer gel, with or without plant extracts, was applied onto a 15-cm^2 surface including the six wounds of each rat twice daily.

The animals were randomly assigned to groups of 6 rats. Two groups served as controls: one group was treated with pure gel only (vehicle control), whereas the other group was not treated at all (absolute control). A third group was treated with 1% salicylic acid gel as positive control. The five remaining groups were treated with gel containing the different plant extracts (CSE-1–CSE-5) in concentrations of 2.5%.

2.5. Statistical analysis

The results are presented as means ± SEM. Data were evaluated statistically by one-way ANOVA, followed by the Dunnett test, by means of the GraphPad Prism 4.03 computer program (San Diego, USA). Statistical significance was accepted at a probability level of $p < 0.05$.

3. Results

The mean time for wound healing without any treatment was 17.1 days, that with the pure vehicle was 17.0 days, and that in the group treated with the positive control was 13.9 days (Fig. 1). The most apolar fraction of the methanol extract (CSE-3) significantly accelerated the wound healing (mean wound-healing time 14.4 days) as compared with the vehicle group; this effect was rather similar to that of the positive control. Extracts CSE-1, CSE-2,

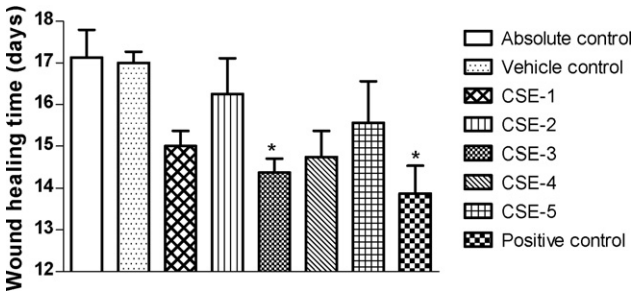

Fig. 1. Effects of different *Centaurea sadleriana* extracts on wound-healing time in rats. The wound-healing time was calculated as the number of days required for 50% of the scabs to separate spontaneously from the animals. $^{*}p < 0.05$ as compared with the vehicle control.

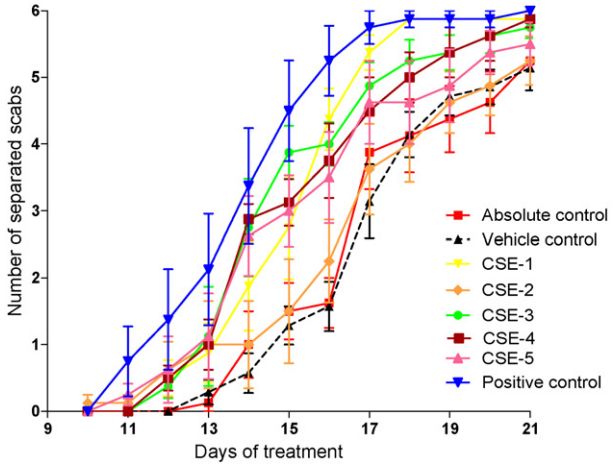

Fig. 2. Number of separated scabs as a function of the treatment duration.

CSE-4 and CSE-5 exhibited more moderate, nonsignificant activities (mean wound-healing time 14.8–16.3 days). The positive effects of the herbal extracts were tendentious throughout the whole experiment (Fig. 2).

4. Conclusions

The present study has demonstrated that the regular topical application of certain *Centaurea sadleriana* extracts accelerates the wound-healing process, confirming the rationale of the traditional ethnomedicinal application of this plant. In view of the characteristics of the experimental setting, the type of the wound and the duration of treatment, primarily the inflammatory and proliferative phases may be influenced. None of the extracts affected the wound healing unfavorably. All the extracts exhibited a beneficial effect, though in most cases statistically not significantly (the only exception was CSE-3). The aqueous extract (CSE-1), prepared similarly to that applied in folk medicine, was moderately efficient. It is not known which of the constituents of *Centaurea sadleriana* are responsible for the wound-healing improvement, but it is plausible that the apolar compounds play a key role in the therapeutic effect.

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